REMARKS

In view of the following Remarks, the Examiner is requested to withdraw the rejection and allow Claims 1, 5-8, 13, 17, 28, and 30-33, the only claims pending and currently under examination in this application.

FORMAL MATTERS:

Claims 27 and 29 are canceled without prejudice.

Claims 1, 13, 28, 30, 31 and 32 are amended. Support for these amendments may be found throughout the specification as originally filed, and more particularly, at page 14, lines 31-33; page 7, lines 28-30; page 10, lines 5-7; and page 16, lines 9-19.

The specification has been amended to introduce an amended Sequence Listing.

No new matter is added. As such, the Examiner is requested to enter the above amendments.

INTERVIEW SUMMARY:

Applicants thank Examiner Shen for the courtesy of conducting a telephone interview on April 1, 2010 with Applicants' representatives Bret Field and Elizabeth Alcamo. Applicants proposed several claim amendments to clarify the language of the claims so as to overcome rejections under 35 U.S.C. §112, second paragraph, and §102. Applicants also proposed claim amendments that would narrow the scope of the claims so as to overcome rejections under 35 U.S.C. §112, first paragraph, for written description and enablement. Examiner Shen indicated that such amendments may be sufficient for overcoming these rejections.

CERTIFICATION REGARDING SEQUENCE LISTING:

I hereby certify that the enclosed Sequence Listing is being submitted under 37 CFR §§ 1.821(c) and (e) in paper and computer readable form (.TXT).

As required by 37 CFR 1.821(f), I hereby state that the content of the paper and computer readable copy of the Sequence Listing, submitted in accordance with 37 C.F.R. §1.821(c) and (e) are the same. The Computer Readable Format (CRF), being submitted under 37 CFR §§ 1.52(e) and 1.824, is formatted on IBM-PC, the operating system compatibility is MS-Windows and the file listing is:

Sequence listing.txt 39KB created March 10, 2010.

I hereby certify that the enclosed submission includes no new matter. The Sequence Listing was prepared with the software PatentIn, and conforms to the Patent Office guidelines.

Applicant respectfully submits that the subject application is in adherence to 37 CFR §§ 1.821-1.825.

REJECTIONS UNDER §112, ¶2

Claims 1, 5-8, 13, 17, and 27-30 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention.

The Examiner asserts that Claim 13 further broadens the scope of Claim 1, and that, as a dependent of Claim 1, the metes and bounds of Claim 13 cannot be determined since two distinct scopes are recited.

Claim 1 as amended recites "an isolated nucleic acid molecule encoding a fluorescent protein, wherein said protein has at least 90% identity with full length SEQ ID NO:10". Thus, Claim 1 is drawn to identity at the amino acid sequence level. Applicants note that, in view of the degeneracy of the genetic code, such a claim provides only a limited indication of sequence identity at the nucleic acid level.

In contrast, as filed in the previous response, Claim 13 recited "a nucleic acid molecule having a sequence that is substantially the same as, or identical to a nucleotide sequence of at least 300 nucleotides in length of the nucleic acid molecule according to Claim 1". Thus, Claim 13 is drawn to identity at the nucleotide sequence level, requiring that the nucleic acid of the claim have complete identity to the nucleic acid of Claim 1 across a span of at least 300 nucleotides in length. Accordingly, Claim 13 is, in fact, narrower in scope than Claim 1. In an effort to clarify the language of the claim, Applicants herein amend Claim 13 to remove the language "--substantially the same as or--" and to recite that the 300 nucleotides in length are 300 contiguous nucleotides of SEQ ID NO:9. As such, the claim is now drawn to a nucleic acid that encodes a protein that has at least 90% identity with full length SEQ ID NO: 10, wherein the nucleic acid comprises a sequence that is identical to a nucleotide sequence of at least 300 contiguous nucleotides in length of SEQ ID NO:9. Thus, Claim 13 is clearly narrower than Claim 1.

The Examiner asserts that Claims 31 and 32 are unclear because, in the absence of recitation of "full length SEQ ID NO:10" in Claim 31 and "full length SEQ ID NO:9" in Claim 32, the breadth of Claims 31 and 32 encompasses any nucleotides sequence having at least 90% identity with any fragment of SEQ ID NO:10 or any fragment of SEQ ID NO:9.

Applicants have amended Claims 31 and 32 to recite "at least 90% identity with full length SEQ ID NO:10" and "at least 90% identity with full length SEQ ID NO:9", respectively.

The Examiner asserts that Claims 27, 28, and 30 are unclear for similar reasons as for Claims 31 and 32, namely, that "full length SEQ ID NO:10" is recited in Claim 1 whereas Claims 27, 28 and 30 recite simply "SEQ ID NO:10" and "SEQ ID NO:9". The Examiner further notes that Claim 27, like Claim 1, recites the 85% identity.

Applicants thank the Examiner for noting the redundancy of Claim 27 with Claim 1 and herein cancel Claim 27. Applicants submit that the language of Claims 28 ("The nucleic acid molecule according to claim 1 which encodes SEQ ID NO:10") and 30 ("The nucleic acid molecule according to claim 1, having a nucleotides sequence comprising SEQ ID NO:9") as filed in the previous response clearly indicates that what is being referred to is full length sequences. However, in an effort to expedite prosecution, Applicants herein amend Claims 28 and 30 to recite "full length SEQ ID NO:10" and "full length SEQ ID NO:9".

The Examiner asserts that Claim 29 is further broadening the scope of Claim 1 because the nucleic acid molecule hybridizes to the nucleic acid of Claim 1 encompasses various fragments of nucleic acid sequence encoding SEQ ID NO:10.

Applicants have canceled this claim. Accordingly, this rejection is moot.

The Examiner asserts that Claim 33 is unclear because Claim 33 depends from Claim 1 and Claim 1 recites "a fluorescent protein" whereas Claim 33 recites "wherein the protein comprises a fluorophore." The Examiner advises the Applicant to clarify on the record with regard to why claim 33 is a further limiting claim in terms of the definition of "a fluorescent protein" recited in claim 1 and the definition of "a fluorophore" recited in Claim 33.

Applicants submit that Claim 33 is included in the pending claims for its recitation of the structural element that provides for the fluorescence activity of the protein of Claim 1. It is the Applicants' position that the presence of a fluorophore is, in fact, implied in Claim 1 by the requirement that the nucleic acid of Claim 1 encode a protein that is fluorescent. Nonetheless, Applicants have provided Claim 33 to recite this structural element, should the recitation of 90% identity to SEQ ID NO:10 be deemed an insufficient structural description by the Office.

In view of the above amendments and remarks, reconsideration and withdrawal of this rejection is respectfully requested.

REJECTIONS UNDER §112, ¶1 – WRITTEN DESCRIPTION

Claims 1, 5-8, 13, 17, 27-30, and 31-33 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

To satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention. See e.g., *Moba, B.V. v. Diamond Automation, Inc.*, 325 F.3d 1306, 1319, 66 U.S.P.Q.2d 1429, 1438 (Fed. Cir. 2003); *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1563, 19 U.S.P.Q.2d 1111, 1116 (Fed. Cir. 1991).

In addition, MPEP 2163 [R5] states:

> The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice (see i)(A), above), reduction to drawings (see i)(B), above), or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus (see i)(C), above). See *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406.

Claim 1 as amended recites "an isolated nucleic acid molecule encoding a fluorescent protein, wherein said protein has at least 90% identity with full length SEQ ID NO:10." Applicants submit that the specification provides a sufficient description of a representative number of nucleic acid molecules that encode a fluorescent protein with at least 90% identity to SEQ ID NO:10 by actual reduction to practice and by disclosure of relevant identifying characteristics such that it would be clear to one of ordinary skill in the art that Applicants were in possession of the claimed genus of nucleic acids.

First, the specification teaches 7 examples of nucleic acids that encode proteins with at least 90% identity to full length SEQ ID NO:10. These include wild type phiYFP (SEQ ID NO:1,

encoding SEQ ID NO:2) and phiYFP mutants Y1 (SEQ ID NO:3, encoding SEQ ID NO:4), M0 (SEQ ID NO:5, encoding SEQ ID NO:6), M1 (SEQ ID NO:7, encoding SEQ ID NO: 8), M1 humanized (SEQ ID NO:9, encoding SEQ ID NO: 10), M1G1 (SEQ ID NO:17, encoding SEQ ID NO:18), and M1C1 (SEQ ID NO:19, encoding SEQ ID NO:20) (p. 25, l. 5 – p. 27, l. 10). Thus, the specification provides a description of a number of nucleic acid molecules that encode a fluorescent protein with at least 90% identity to SEQ ID NO:10 by actual reduction to practice.

Second, the specification teaches the relevance of the structure-function relationship in GFP and Anthozoan fluorescent proteins to the relationship between structure and fluorescence in the disclosed proteins (p. 1, l. 13-26; Figure 1). Additionally, it was well known in the art which amino acids encode for the structures that provide for fluorescence in GFP and Anthozoan fluorescent proteins and thus should be conserved, and, conversely, which amino acid residues may be modified without losing fluorescence; see, e.g., Yang et al. ((1996) Nat. Biotech 14:1246-51); Ormo et al. ((1996) Science 273:1392-95); Matz et al. ((1999) Nat Biotech 17:969-973); Heim et al. ((1996) Current Biol. 6:178-182); Siemering et al. ((1996) Current Biol. 6(12):1653-63); Yang et al. ((1998) J Biol Chem 273(14):8212-8216); Wiedenmann et al. ((2000) PNAS 97(26):14091-6); Bevis et al. ((2002) Nat. Biotechnol 20(1):83-7); Campbell et al. ((2002) PNAS 99(12):7877-82); and Shaner et al. ((2004) Nat Biotechnol 22(12):1567-72), all of the record and discussed in greater detail in previous responses. Thus, in addition to disclosing multiple examples by actual reduction to practice, Applicants have also disclosed relevant identifying characteristics, i.e. functional characteristics that are known in the art to correlate with structure.

Finally, Applicants note that Example 11B of the Written Description Guidelines published March 25, 2008 (p. 41-42) teaches that, when an art-recognized structure-function correlation is present, a claim that encompasses "a genus of nucleic acids that encode any polypeptide having structural identity of 85% to a claimed sequence, wherein the polypeptide additionally has activity Y" is sufficiently supported by a disclosure of reduction to practice of one species. This example is reproduced below for the Examiner's convenience:

Claim 2

Claim 2 encompasses a genus of nucleic acids that encode the polypeptide of SEQ ID NO: 2 and those that encode any polypeptide having 85% structural identity to SEQ ID NO: 2, wherein the polypeptide additionally has activity Y.

The specification, however, discloses the reduction to practice of only a single species that encodes SEQ ID NO: 2 and has activity Y, *i.e.*, SEQ ID NO: 1. There are no other drawings or structural formulas disclosed of a nucleic acid that encodes either (i) SEQ ID NO: 2 or (ii) a polypeptide with 85% sequence identity to SEQ ID NO: 2 wherein the polypeptide also has activity Y.

The disclosure of SEQ ID NO: 2 combined with the knowledge in the art regarding the genetic code would have put one in possession of the genus of nucleic acids that encode SEQ ID NO: 2. Further, with the aid of a computer, one could list all of the nucleic acid sequences that encode a polypeptide with at least 85% sequence identity to SEQ ID NO: 2. However, the specification fails to teach which of the nucleic acid sequences that encode a polypeptide with at least 85% sequence identity to SEQ ID NO: 2 encode a polypeptide having the required activity Y.

Practice Note

This example deals only with the written description analysis of the claimed nucleic acids. Enablement issues that may be raised by the recited facts are not addressed here, but should be considered during examination. A separate rejection for nonenablement should be made when appropriate.

Nonetheless, the specification identifies two domains responsible for activity Y, *i.e.*, a binding domain and catalytic domain. The specification also predicts that conservative mutations in these domains will result in a protein having activity Y. Although all conservative amino acid substitutions in these domains will not necessarily result in a protein having activity Y, those of ordinary skill in the art would expect that many of these conservative substitutions would result in a protein having the required activity. Further, amino acid substitutions outside of the two identified functional domains are unlikely to greatly affect activity Y. Thus, a correlation exists between the function of the claimed protein and the structure of the disclosed binding and catalytic domains. Consequently, there is information about which nucleic acids can vary from SEQ ID NO: 1 in the claimed genus of nucleic acids and still encode a polypeptide having activity Y. Based on the applicant's disclosure and the knowledge within the art, those of ordinary skill in the art would conclude that the applicant would have been in possession of the claimed genus of nucleic acids based on the disclosure of the single species of SEQ ID NO: 1.

Conclusion:

The specification satisfies the written description requirement of 35 U.S.C. 112, first paragraph, with respect to the scope of claim 2.

Surely, if 85% identity satisfies the written description requirement under such circumstances, 90% identity as recited by the pending claims must also satisfy the written description requirement.

Thus, Applicants maintain that the specification in view of the art provides a sufficient description of a representative number of species by both actual reduction to practice and by disclosure of relevant, identifying characteristics, i.e. the functional characteristic of fluorescence coupled with a known correlation in the art between fluorescence and structure, to demonstrate that Applicants were in possession of the claimed invention.

In response, the Examiner acknowledges that the state of the art does provide information pertaining to GFP as cited by Applicant (p. 14, l. 14-15). The Examiner then asserts that "However, Applicant is reminded that the claimed invention is directed to SEQ ID NO:10, which is neither a GFP nor a dsRFP. In this regard, it is noted that the excitation–emission spectra of phiYFP are distinct from the excitation-emission spectra of GFP and dsRFP, and the identical sequences between GFP, dsRFP and phiYFP are only 12.8% as Applicant states in the remarks. Accordingly, there is no evidence on the record how an artisan can base on the teachings regarding GFP and dsRFP to determine the structure-function relationship of the genus of sequences of phiYFP claimed by Applicant." (p. 14, l. 22-28) Finally, the Examiner asserts that "SEQ ID NO:10 is a 234-amino acid long polypeptide and is a humanized version of the phiYFG-M1, which is the mutant form of phiYFP generated by random mutagenesis of phiYFP. The mutation was not based on any known structure-function relationship of phiYFP." (p. 14, l. 28 - 15, l. 3)

Applicants acknowledge that, indeed, phiYFP is not GFP or dsRED. However, Applicants note that phiYFP *is* a fluorescent protein and that, as such (and as taught by the specification), the knowledge of the structural elements that provide for the fluorescence of GFP and dsRED *are relevant* to the knowledge of the structure of phiYFP. Applicants note that it appears that the Applicants' assertions in the previous response were misunderstood: the statement made by Applicants on 12.8% identity was made with regard to residues that are conserved among *all* fluorescent proteins disclosed in the subject application and GFP, i.e. in a single alignment, and was provided as evidence that a significant amount of variation in the protein sequence may be tolerated without loss of fluorescence activity. In fact, the wild type

phiYFP fluorescent protein of the subject invention shares about 50% identity with GFP (see figure 1, page 4, lines 20-23, and page 25, lines 16-18), and thus would be immediately recognized by one of ordinary skill in the art upon its isolation to be a member of the fluorescent protein family.

Furthermore, Applicants note that the claims recite no limitations regarding the excitation or emission spectra of the fluorescent protein encoded by the claimed nucleic acid, only that the nucleic acid encodes a protein that is fluorescent. Thus, mutations in SEQ ID NO:10 that modulate excitation and/or emission spectra, brightness, or any other characteristic of the fluorescence of the encoded protein are, in fact, *encompassed by the pending claims*.

Finally, Applicants assert that it is inappropriate to assume from the experimental approach used to arrive at the mutants disclosed in the present application that the Applicants had no knowledge of a relationship between the structure of the phiYFP protein and its function. The teachings of the specification clearly demonstrate that, contrary to the Examiner's assertions, Applicants *did* have an understanding of a relationship between the disclosed wild type proteins and GFP and dsRED, of the relationship between the structure of GFP and dsRED and their fluorescence activity, and hence of the relationship between the structures of the disclosed wild type proteins and the fluorescence activity of these wild type proteins. Furthermore, there are many reasons why one would choose the experimental approach chosen by the Applicants to arrive at the disclosed mutants, e.g. a desire to perform rapid and high-throughput screening, the availability (or lack thereof) of reagents required for screening versus site-directed mutagenesis, etc. Moreover, some of the mutants disclosed were, in fact, arrived at by site-directed mutagenesis; see, e.g. the M1 mutant (p. 26, l. 8-15) and the M1G1 and M1C1 mutants (p. 27, l. 1-10). Accordingly, the fact that random mutagenesis was used to identify some of the disclosed mutants provides *no indication whatsoever* of the extent of the Applicants' knowledge of the relationship between the structure of phiYFP and its fluorescence.

Thus, Applicants submit that they have provided a written description such the one of ordinary skill in the art would reasonably conclude that the inventor had possession of the claimed invention. Reconsideration and withdrawal of the rejection is requested.

REJECTIONS UNDER §112, ¶1 - ENABLEMENT

Claims 1, 5-8, 13, 17, 27-30, and 31-33 are rejected under 35 U.S.C. 112, first paragraph, because the specification allegedly does not reasonably provide enablement for (1) any isolated nucleic acid molecule encoding a fluorescent protein other than SEQ ID No. 9 that encodes a fluorescent protein consisting of SEQ ID No. 10, or (2) any vector/cell/kit comprising any isolated nucleic acid molecule encodes a fluorescent protein other than SEQ ID NO 9 that encodes a fluorescent protein consisting of SEQ ID No. 10.

With respect to enablement, courts have held that: "[t]he test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation." *United States v. Telectronics, Inc.,* 8 USPQ 2d 1217, 1233 (Fed. Cir. 1988), *cert. denied*, 490 U.S. 1046 (1989). See also *Genentech, Inc. v. Novo Nordisk*, 42 USPQ 2d 1001 (Fed. Cir. 1997), *cert. denied*, 522 U.S. 963 (1997); *Scripps Clinic and Research Foundation v. Genentech, Inc.*, 18 USPQ 2d 1001 (Fed. Cir. 1991).

In making this rejection, the Examiner asserts that "[t]he basis of this scope of enablement is hinged on the lack of enabling support on the structure/function relationship to make and use any isolated nucleic acid molecule comprising nucleotide sequences encoding a fluorescent protein having at least 85% identity with SEQ ID NO:10 recited in independent Claim 1, and further broadening scope of nucleic acid molecules encompassed by dependent claims 13 and 29", applying the criteria set forth in *Wands* to arrive at this conclusion.

Applicants submit that, contrary to the Examiner's assertions and discussed in greater detail below, sufficient support on the structure/function relationship to make and use the nucleic acid molecules of the pending claims is, in fact, provided by the specification and the art and that, as such, the pending claims are sufficiently enabled.

Guidance and working examples provided

"The Examiner asserts that the specification does not provide any guidance regarding the structure-function correlation of phiYFP in terms of which amino acids are necessary and sufficient for phiYFP to be a fluorescent protein. It would require undue experimentation for an artisan to determine which amino acids are necessary and sufficient for phiYFP-M1 (i.e. the

claims SEQ ID NO:10) to be a fluorescent protein to support the breadth of the claims." (p. 19, l. 10-14)

Newly amended Claim 1 recites "An isolated nucleic acid molecule encoding a fluorescent protein, wherein said protein has at least 90% identity with full length SEQ ID NO: 10." Applicants submit that the specification in view of the art teaches a wealth of guidance and working examples that the ordinary skilled artisan would be able use to determine which amino acids are necessary and sufficient in SEQ ID No.10 to maintain fluorescence so as to identify other nucleic acids of the claimed genus.

As discussed above and in previous responses, the specification teaches 7 examples of nucleic acids that encode proteins with at least 90% identity to full length SEQ ID NO:10. These include wild type phiYFP (SEQ ID NO:1, encoding SEQ ID NO:2) as well as phiYFP mutants Y1 (SEQ ID NO:3, encoding SEQ ID NO:4), M0 (SEQ ID NO:5, encoding SEQ ID NO:6), M1 (SEQ ID NO:7, encoding SEQ ID NO: 8), M1 humanized (SEQ ID NO:9, encoding SEQ ID NO: 10), M1G1 (SEQ ID NO:17, encoding SEQ ID NO:18), and M1C1 (SEQ ID NO:19, encoding SEQ ID NO:20) (p. 25, l. 5 – p. 27, l. 10).

Furthermore, the specification in view of the art provides a wealth of guidance and working examples for methods of arriving at other nucleic acids that encode proteins with at least 90% identity to full length SEQ ID NO:10. For example, methods of isolating wild type proteins having a sequence identity of at least 90% with a known protein, e.g. degenerate PCR and BLAST searching, are well understood in the art, and thus, one of ordinary skill in the art would know how to identify other nucleic acid sequences that encode wild type fluorescent proteins having a sequence identity of at least 90% with SEQ ID NO:10. Likewise, the degeneracy of the genetic code is well understood in the art, and thus, one of ordinary skill in the art would know how to design a multitude of other nucleic acid sequences that would also encode these wild type fluorescent proteins. Likewise, the specification in view of the art teaches methods of identifying which amino acids can be substituted in fluorescent proteins so as to retain fluorescence. For example, the specification teaches the relevance of the relationship between the structure and fluorescence of GFP and Anthozoan fluorescent proteins to the relationship between the structure and fluorescence of the disclosed proteins (p. 1, l. 13-26; Figure 1). The art teaches which amino acids encode the structure that provided for fluorescence and thus should be conserved, and, conversely, which amino acid residues could

be modified without losing fluorescence; see, e.g., Yang et al. ((1996) Nat. Biotech 14:1246-51); Ormo et al. ((1996) Science 273:1392-95); Matz et al. ((1999) Nat Biotech 17:969-973); Heim et al. ((1996) Current Biol. 6:178-182); Siemering et al. ((1996) Current Biol. 6(12):1653-63); Yang et al. ((1998) J Biol Chem 273(14):8212-8216); Wiedenmann et al. ((2000) PNAS 97(26):14091-6); Bevis et al. ((2002) Nat. Biotechnol 20(1):83-7); Campbell et al. ((2002) PNAS 99(12):7877-82); and Shaner et al. ((2004) Nat Biotechnol 22(12):1567-72), all of the record and discussed in greater detail in previous responses. Thus, the artisan would be enabled to predict exactly which amino acids of SEQ ID NO:10 are conserved and should not be mutated versus those which are not conserved and could be mutated so as to retain fluorescence activity. Additionally, the specification teaches methods of testing these predictions, by teaching methods of making mutant nucleic acids encoding mutant proteins (p. 8, l. 21-p. 9, l. 9; see also p. 26, l. 8-11), and of testing these mutant nucleic acid, for example by transfecting the nucleic acids into cells in culture, waiting 20 hours, and imaging the cells on a fluorescence microscope (p. 29, l. 7-16). Thus, one of ordinary skill in the art would know how to isolate or design a multitude of other nucleic acid sequences that would encode fluorescent proteins encompassed by the pending claims.

Accordingly, Applicants maintain that the specification in view of the art provides a reasonable amount of guidance and working examples on the relationship between the structure of the disclosed proteins and their function as fluorescent molecules that one of ordinary skill in the art would be able to identify other species of the claimed genus without undue experimentation.

In response, the Examiner acknowledges that the status of art does provide information pertaining to GFP as cited by Applicant (p. 23, l. 1-2). The Examiner then asserts that "However, Applicant is reminded that the claimed invention is directed to SEQ ID NO:10, which is neither a GFP nor a dsRFP. In this regard, it is noted that the excitation–emission spectra of phiYFP are distinct from the excitation-emission spectra of GFP and dsRFP, and the identical sequences between GFP, dsRFP and phiYFP are only 12.8% as Applicant states in the remarks. Accordingly, there is no evidence on the record how an artisan can base on the teachings regarding GFP and dsRFP to determine the structure-function relationship of the genus of sequences of phiYFP claimed by Applicant." (p. 23, l. 9-15) Finally, the Examiner asserts that "SEQ ID NO:10 is a 234-amino acid long polypeptide and is a humanized version of the phiYFG-M1, which is the mutant form of phiYFP generated by random mutagenesis of

phiYFP. The mutation was not based on any known structure-function relationship of phiYFP." (p. 23, l. 15-19)

Applicants acknowledge that, as discussed above, phiYFP is not GFP or dsRED. However, Applicants submit that phiYFP *is* a fluorescent protein and that, as such (and as taught by the specification), the knowledge of the structural elements that provide for the fluorescence of GFP and dsRED *are relevant* to the knowledge of the structure of phiYFP. Applicants note that it appears that Applicants' assertions in the previous response were misunderstood: the statement made by Applicants on 12.8% identity was made with regard to amino acids that are conserved among *all* fluorescent proteins disclosed in the subject application and GFP, i.e. in a single alignment, and was provided as evidence that much variation may be tolerated in the protein sequence without loss of fluorescence activity. In fact, the wild type phiYFP fluorescent protein of the subject invention shares about 50% identity with GFP (see figure 1, page 4, lines 20-23, and page 25, lines 16-18), and thus would be immediately recognizable by one of ordinary skill in the art upon its isolation to be a member of the fluorescent protein family.

Furthermore, Applicants note that the claims recite no limitations regarding the excitation or emission spectra of the fluorescent protein encoded by the claimed nucleic acid, only that the nucleic acid encodes a protein that is fluorescent. Thus, mutations in SEQ ID NO:10 that modulate excitation and/or emission spectra, brightness, or any other characteristic of the fluorescence of the encoded protein are, in fact, *encompassed by the pending claims*. Accordingly, no particular guidance is required on, for example, how to make mutants that retain a particular excitation and/or emission spectra.

Finally, Applicants assert that it is inappropriate to assume from the experimental approach used to arrive at the mutants disclosed in the present application that the Applicants had no knowledge of a relationship between the structure of the phiYFP protein and its function. The teachings of the specification clearly demonstrate that, contrary to the Examiner's assertions, Applicants *did* have an understanding of a relationship between the disclosed wild type proteins and GFP and dsRED, of the relationship between the structures of GFP and dsRED and GFP and dsRED fluorescence activity, and hence of the relationship between the structures of the disclosed wild type proteins and the fluorescence activity of these wild type proteins. Furthermore, there are many reasons why one would choose the experimental

approach chosen by the Applicants to arrive at the disclosed mutants, e.g. a desire to perform rapid and high-throughput screening, the availability (or lack thereof) of reagents required for screening versus site-directed mutagenesis, etc. Finally, some of the mutants disclosed in the present application were, in fact, arrived at by site-directed mutagenesis; see, e.g. the M1 mutant (p. 26, l. 8-15) and the M1G1 and M1C1 mutants (p. 27, l. 1-10). Accordingly, the fact that random mutagenesis was used to identify some of the disclosed mutants *is no indication whatsoever* of the extent of the Applicants' knowledge of the relationship between the structure of phiYFP and its fluorescence.

Thus, Applicants maintain that the specification in view of the art provides a reasonable amount of guidance and working examples on the relationship between the structure of the disclosed proteins and their function as fluorescent molecules that one of ordinary skill in the art would be able to identify other species of the claimed genus without undue experimentation.

State of the art, level of predictability in the art

The Examiner asserts that "in the art, it is unpredictable how variations of sequences in a given fluorescent protein would affect its function as a fluorescent protein. For instance, Shagin et al. teaches that homologs of the green fluorescent protein, including the recently described GFP-like domains of certain extracellular matrix proteins in bilateral organism, are remarkably similar at the protein level, yet they often perform totally unrelated functions, thereby warranting recognition as a superfamily." (p. 19, l. 15-20; p. 24, l.)

Applicants submit that Shagin et al., in fact, supports the Applicants assertions of the predictability in the art of fluorescent proteins with regard to the relationship between structure and function and the predictions that may be made based upon this relationship. Shagin et al. teaches a class of proteins from bilateral organisms call G2FP proteins that have a GFP-like domain but that perform totally unrelated functions to GFP proteins (i.e. they are not fluorescent or colored, but instead serve as protein-binding modules that participate in control of the extracellular matrix formation during development) (abstract, l. 1-3, and p. 842, col. 1, l. 29-34). Shagin et al. teaches that the G2FP proteins should be considered structural homologs of GFP because they share the GFP-like "beta-can" fold domain (p. 844, col. 2, l. 26-29). However, no mention is made of conservation of a fluorophore domain, a three-amino acid domain which, as discussed above, is known in the art (see, e.g. Matz et al.) to be necessary for fluorescence. Furthermore, Hopf et al., which is cited by Shagin et al. as the art that first demonstrating this

conservation of the beta-can domain between the G2FP proteins from bilateral organisms and the GFP family of fluorescent proteins, also teaches no fluorophore; rather, Hopf et al. teaches that the G2FP protein under study ("Nidogen") comprises an EGF domain and a beta-can domain. See, for example, the abstract, provided below:

Nidogen, an invariant component of basement membranes, is a multifunctional protein that interacts with most other major basement membrane proteins. Here, we report the crystal structure of the mouse nidogen-1 G2 fragment, which contains binding sites for collagen IV and perlecan. The structure is composed of an EGF-like domain and an 11-stranded β-barrel with a central helix. The β-barrel domain has unexpected similarity to green fluorescent protein. A large surface patch on the β-barrel is strikingly conserved in all metazoan nidogens. Site-directed mutagenesis demonstrates that the conserved residues are involved in perlecan binding.

Thus, the teachings of Shagin et al. are consistent with what one of ordinary skill in the art would already know from the art, namely, the importance of the fluorophore and the amino acids that comprise it, and which amino acids should be conserved when varying the sequence in a given fluorescent protein so as to not affect its function as a fluorescent protein. Accordingly, and contrary to the Examiner's assertions, Shagin et al. actually demonstrates the predictability in the art of fluorescent proteins.

Thus, Applicants maintain that the state of the relevant art with regard to predicting and validating the residues of fluorescent proteins from proteins having 90% identity to SEQ ID NO:10 that may be mutated while retaining fluorescence activity is well-developed. Likewise, the art with regard to predicting and validating the residues of chromo- and fluorescent proteins having 90% identity to SEQ ID NO:10 that can be mutated so as to retain fluorescent activity is also highly predictable.

Therefore, the guidance and working examples provided by the specification and the art in view of the well developed state of the relevant art and the high level of predictability in the art provide suitable support for the claimed breadth, and that, as such, one of ordinary skill in the art would be able to practice the claimed invention without undue experimentation.

In view of these remarks, reconsideration and withdrawal of the rejection is requested.

REJECTIONS UNDER §102

I. Claims 1, 5-8, 13, 17, and 27-30 are rejected under 35 U.S.C. 102(e) as being anticipated by Baubet et al. (Baubet et al. US 2008/0213879, publication date 09/04/2008, Division of US 6,936,475, which is a Continuation of PCT/EP01/07057, WO 2001/092300, filed on 06/01/2001).

A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. *Verdegaal Bros. v. Union Oil of California*, 814 F.2d 628, 631, (Fed. Cir. 1987).

The standard for anticipation under section 102 is one of strict identity. An anticipation rejection requires a showing that each limitation of a claim be found in a single reference, *Atlas Powder Co. v. E.I. DuPont de Nemours & Co.,* 224 U.S.P.Q. 409, 411 (Fed. Cir. 1984). Further, an anticipatory reference must be enabling, see *Akzo N.V. v. United States Int'l Trade Comm'n* 808 F.2d 1471, 1479, 1 U.S.P.Q.2d 1241, 1245 (Fed. Cir. 1986), *cert denied*, 482 U.S. 909 (1987), so as to place one of ordinary skill in possession of the claimed invention. To anticipate a claim, a prior art reference must disclose every feature of the claimed invention, either explicitly or inherently. *Glaxo v. Novopharm, Ltd.* 334 U.S. P.Q.2d 1565 (Fed. Cir. 1995).

Claim 1 as amended recites "an isolated nucleic acid molecule encoding a fluorescent protein, wherein said protein has at least 90% identity with full length SEQ ID NO: 10." Claim 13 as amended recites "The nucleic acid molecule according to claim 1, wherein said nucleic acid molecule comprises a sequence that is identical to a nucleotide sequence of at least 300 contiguous nucleotides in length of SEQ ID NO:9."

Applicants submit that Baubet et al. does not disclose a nucleic acid molecule that encodes a fluorescent protein having at least 85% identity with full length SEQ ID NO: 10 as recited in Claim 1, or a nucleic acid that comprises a sequence that is identical to a nucleotide sequence of at least 300 contiguous nucleotides of SEQ ID NO:9, as recited in Claim 13. Rather, as evidenced by the amino acid and nucleotide alignments provided by the Examiner in the Final Office Action pages 28-31 and 31-37, respectively, Baubet et al. discloses nucleic acids that encode polypeptides with only 50.5% identity with full length SEQ ID NO:10 and nucleotide sequences that are, at best, identical to a nucleotide sequence of only 20 contiguous nucleotides of SEQ ID NO:9. Thus, Baubet et al. does not anticipate the pending claims.

In making this rejection, the Examiner has cited alleged indefiniteness in the claims (see the rejection under 35 U.S.C. §112, second paragraph, above) and, in view of this alleged indefiniteness, interpreted the limitation in Claim 13 of "at least 300 nucleotides in length of the nucleic acid molecule of Claim 1" to read on identical nucleotide sequences that are not

necessarily continuous, hence requiring an identity of any 100 amino acid residues (which correspond to 300 nucleotides) to SEQ ID NO:10 (full length 234 amino acid residues), i.e. 42.7% identity (p. 26, l. 14-19). Furthermore, to encompass the alleged breadth of the dependent Claim 31, the Examiner has interpreted the limitation in Claim 1 of "an isolated nucleic acid molecule encoding a fluorescent protein, wherein said protein has at least 85% identity with full length SEQ ID NO: 10" as meaning "a fluorescent protein having a fragment with at least 9 out of 10 amino acid residues identical to the sequence of the full length SEQ ID NO:10 (i.e. 90% identity recited in claim 31)." (p. 27, l. 2-5)

In an effort to expedite prosecution and without agreeing to the correctness of the rejection, Applicants have amended Claim 13 to recite that the 300 nucleotides are contiguous nucleotides of SEQ ID NO:9, and have amended Clams 28, 30, 31 and 32 such that the claims read on the full length sequence recited therein. In view of these amendments, amended Claim 1 is now clearly drawn to a nucleic acid molecule encoding a fluorescent protein with 90% identity with full length SEQ ID NO: 10, and Claim 13 is now clearly drawn to a nucleic acid comprising a sequence that is identical to a nucleotide sequence of at least 300 contiguous nucleotides in length of SEQ ID NO:9. As such, and as discussed above, Baubet et al. does not anticipate the pending claims.

In view of the claim amendments and remarks above, reconsideration and withdrawal of the rejection is requested.

II. Claims 1, 5-8, 13, 17, 27-30, and 31-33 are rejected under 35 U.S.C. 102(b) as being anticipated by Baubet et al. (PCT/EP01/07057, WO 2001/092300, filed on 06/01/2001).

As discussed above, Claim 1 as amended recites "an isolated nucleic acid molecule encoding a fluorescent protein, wherein said protein has at least 90% identity with full length SEQ ID NO: 10." Claim 13 as amended recites "The nucleic acid molecule according to claim 1, wherein said nucleic acid molecule comprises a sequence that is identical to a nucleotide sequence of at least 300 contiguous nucleotides in length of SEQ ID NO:9."

As discussed above, Applicants submit that Baubet et al. does not disclose a nucleic acid molecule that encodes a fluorescent protein having at least 85% identity with full length SEQ ID NO: 10, as recited in Claim 1, or a nucleic acid that comprises a sequence that is

identical to a nucleotide sequence of at least 300 contiguous nucleotides of SEQ ID NO:9, as recited in Claim 13. Rather, as evidenced by the amino acid and nucleotide alignments provided by the Examiner in the Final Office Action pages 28-31 and 31-37, respectively, Baubet et al. discloses nucleic acids that encode polypeptides with only 50.5% identity with full length SEQ ID NO:10 and nucleotide sequences that are, at best, identical to a nucleotide sequence of only 20 contiguous nucleotides of the nucleic acid molecule of Claim 1. Thus, Baubet et al. does not anticipate the pending claims.

In making this rejection, the Examiner has applied the same claim interpretation as that set forth in the rejection under 35 U.S.C. §102(e), reviewed above. A discussed above, Applicants have amended Claim 13 to recite that the 300 nucleotides are contiguous nucleotides of SEQ ID NO:9, and have amended Clams 28, 30, 31 and 32 such that the claims read on the full length sequence recited therein. In view of these claim amendments, amended Claim 1 is now clearly drawn to a nucleic acid molecule encoding a fluorescent protein with 90% identity with full length SEQ ID NO: 10, and Claim 13 is now clearly drawn to a nucleic acid comprising a sequence that is identical to a nucleotide sequence of at least 300 contiguous nucleotides in length of SEQ ID NO:9. As such, and as discussed above, Baubet et al. does not anticipate the pending claims.

In view of the claim amendments and remarks above, reconsideration and withdrawal of the rejection is requested.

CONCLUSION

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number EURE-006.

Respectfully submitted,
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